PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Are low levels of serum bicarbonate associated with risk of progressing to impaired fasting glucose/diabetes? A single-center prospective cohort study in Beijing, China
AUTHORS	Li, Sen; Wang, Ying-Ying; Cui, Jing; Chen, Dong-Ning; Li, Yu; Xin, Zhong; Xie, Rong-Rong; Cao, Xi; Lu, Jing; Yang, Fang-Yuan; Yang, Jin-Kui

VERSION 1 – REVIEW

REVIEWER	M Dobre UHCMC, USA
REVIEW RETURNED	12-Sep-2017

GENERAL COMMENTS	Number of IFG events by groups of bicarbonate should be provided. Also please include number of events in the Table 2. Authors should provide explanation why > 28 mmol/L group was chosen, as reference. Depending on lab, normal values for bicarbonate range somewhere between 23 and 30. There are numerous studies suggesting adverse outcomes at high levels of serum bicarbonate. Maintaining serum bicarbonate above 28 certainly reaches the alkalosis range with its negative consequences, unless there is compensation for severe respiratory acidosis. If available, medication that can influence both the glucose level and serum bicarbonate should be added as co-factors in the analyses (diuretics, beta-blockers, sodium bicarbonate, etc). Proteinuria can occur early in CKD, before creatinine increase, and there is a high association between proteinuria and bicarbonate. The authors should add data on and adjust for proteinuria. One would expect an inverse association between bicarbonate and creatinine which is not the finding here. The authors should provide some explanation in the discussion section. Single measurement of bicarbonate and glucose is also a limitation and should be discussed. What was the role of liver ultrasound? It does not seem to be used in any of the analyses. The relevance of ROC analysis is difficult to interpret. One would rarely perform a test for serum bicarbonate and hope to predict future IGT, where there are so many other more significant predictors of developing diabetes, including age or serum glucose level. This is also seen in the relatively low sensitivity and specificity of the test. Correct unit for creatinine should be included in Methods (page 4) Please check grammar and spelling.

REVIEWER	Maria J Redondo

	Baylor College of Medicine, USA
REVIEW RETURNED	23-Sep-2017

GENERAL COMMENTS	Li et al set out to study the relationship between serum bicarb levels and the risk of progression to impaired fasting glucose or diabetes. This research is significant, novel and well conducted, although important mechanistic information is missing. My major comments relate to the analytical methods and presentation of results. - Subjects and Methods: Please indicate how many participants were still followed at each time point. Also, the schedule of visits is not clearly described and easy to miss by the reader. Please clarify how often, time intervals, number of visits and number (%) of participants at each visit. For each of those questions, please modify the manuscript accordingly. - Related to the above, line 7 indicates "65.6% who completed the follow-up physical examination": Does this refer to finishing all the visits, or being followed by the 6-year time? Please clarify follow-up time and completion rates. Please modify the manuscript accordingly. - Please compare available characteristics between those who completed follow-up and those who were not. Also, please address if they could have been lost to follow-up due to death or other complications of diabetes, and therefore be a group enriched for progressors. If the latter is true, by analyzing only the ones who did complete follow-up, the actual progression to IFG/DM would be underestimated. Please modify the manuscript accordingly and/or discuss in limitations of the study. - Analytical methods: Why were time-to-event analytical methods not used? Given that the follow-up time was different for the participants, time-to-event methods would have allowed to take this into account and maximize the use of available information. Please explain and modify the analysis accordingly. - The authors refer to IFG throughout the manuscript while they are referring to IFG or diabetes. This should be made clear to the reader in the text and abstract. Please refer to "IFG or DM" or "IFG/DM" or any other nomenclature or abbreviation that would be clear to the reader what progression meant in this study. - Lack of mechanistic information is an important limitation of the study, both for the mechanisms of differences in levels of bicarb among participants, and the effect of bicarb on progression. Is there any addtitional information that could help lessen this limitation? For example, other markers of pancreatic insufficiency? - why was dyastolic pressure not included in the models along with systolic? If there is any analytical issue, can mean blood pressure be used instead for adjustment? Why were lipids not included? In general, how were the variables selected to be included or excluded from the multivariable analyses? Please explain in your response, clarify the methods in the manuscript and modify the analysis accordingly. - Results; Please provide the distribution of bicarb levels (median, mean, SD, IQR, range) in your study population as well as the normal reference values in your lab. - Please offer plausible explanations for the lower bicarb levels in individuals with lower FPG, serum creat, TC, TG and LDL at baseline. - Please include the percentile that 26.1 corresponds to in your distribution. - Limitations of the study, besides the ones mentioned, include lack of data to shed light on the mechanism of the association between

	low bicarb and progression (e.g. no data on insulin resistance markers or insulin secretion) and on why the inter individual differences in bicarb (e.g. other markers of pancreatic insufficiency). Other limitations are the seizable proportion of missed to follow-up participants; adjustment for confounder variables may have been incomplete and this finding needs validation in an independent cohort to prove reproducibility. Please update the limitaition section of the discussion as well as the paragraph right after the abstract. - The authors indicate that "data from multicenter" is needed. However, validation could come not only from a multicenter study but from any other independent cohort(s). - Discussion: My interpretation of the findings is that low bicarb is a necessary but not sufficient cause to progress to IFG/DM. Bicarb above a certain level is protective 98% (NPV). Are those statements correct? If so, please clarify in the discussion. I find that more remarkable that a mild increase in the risk conferred by lower carb. Please clarify to this reader and modify discussion if appropriate. - Where medications considered as a potential confounder? Other potential confounders? Please explain and modify the discussion (i.e. add as limitation) accordingly. - Discussion needs to explain the baseline associations that were unexpected (e.g. some of them are inverse to the expected). - DKA also impairs beta-cell function. This work is consistent with that previous observation. Please add to the discussion if appropriate or explain why is not in your response. - MINOR --- The study needs English edits. A few examples are: line 11, page 3: a large 'amount' of participants (please replace with 'number') --- Ultrasound is mentioned in line 13, page 3 but not in the paper. Please reconcile. If not analyzed in this study, please delete. --- line 15-16, page 3, needs English revision ----there are multiple other instances. - page 4, line 5: 'intermediate hyperglycemia' is an unusual name. Please consider replacing with 'pre-diabetes' - "the" public health, extra "the" line 7-8, page 4 - results, line 43-46, page 6,: Table S1 shows that participants..." is a repeat of previous information. - Lines 53-54, page 8,: "Moreover, fasting hyperglycemia..." Please clarify the meaning of this sentence and rephrase accordingly in the manuscript. - Line 17-20 , page 10: the sentence "although HbA1c determination..." is unclear to this reader. Please clarify in your response and rephrase accordingly in the manuscript.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: M Dobre
Institution and Country: UHCMC, USA
Competing Interests: none

Number of IFG events by groups of bicarbonate should be provided. Also please include number of events in the Table 2.
Thank you for the suggestion. The number of IFG events by groups of bicarbonate has been added into the revised manuscripts (Page 7, line 1) and included in the Table 2 as well.

Authors should provide explanation why > 28 mmol/L group was chosen, as reference. Depending on lab, normal values for bicarbonate range somewhere between 23 and 30. There are numerous studies suggesting adverse outcomes at high levels of serum bicarbonate. Maintaining serum bicarbonate above 28 certainly reaches the alkalosis range with its negative consequences, unless there is compensation for severe respiratory acidosis.
As for the reviewer's concern, we added reference in the METHOD of revised manuscripts (Page 5, line 27, Ref. 15). Paul Chubb et al. demonstrated that serum bicarbonate was a significant independent predictor of coronary heart disease. In this research , baseline characteristics of patients were categorized by quintile of serum bicarbonate with Q4 28 mmol/L and Q5≧29 mmol/L. They found that the risk of an incident CHD event decreased by 5% for each 1 mmol/L increase in serum bicarbonate. Besides, the normal reference level of bicarbonate in our lab ranges from 24.2 to 30.7 mmol/L, the participants only with creatinine ≤106.0 μmol/L were chosen to take part in the study, and ultrasonography was performed to avoid renal or hepatic diseases. Therefore, all the groups of participants with different serum bicarbonate were healthy at the starting point.

If available, medication that can influence both the glucose level and serum bicarbonate should be added as co-factors in the analyses (diuretics, beta-blockers, sodium bicarbonate, etc). Proteinuria can occur early in CKD, before creatinine increase, and there is a high association between proteinuria and bicarbonate. The authors should add data on and adjust for proteinuria.
Thank you for this comment. Medication was also concerned by our researchers. All the participants received a medical history questionnaire before and annually during the research, which includes the past history and the drugs that they routinely accepted. Those who were taking medicines that might influence the glucose or serum bicarbonate were excluded. Therefore, we did not add medication as co-factors in the analyses. Similarly, participants with protein, occult blood, or ketones in the routine urine test were also excluded.

One would expect an inverse association between bicarbonate and creatinine which is not the finding here. The authors should provide some explanation in the discussion section.
We are appreciative of the reviewer's suggestion. The mean value of creatinine increased with the rise of serum bicarbonate, however, the creatinine can be influenced by many other factors such as the diet or activities. The association between bicarbonate and creatinine needs to be validated by another logistic regression statistical analysis. In addition, the main point of this study is to focus on the association between glucose and serum bicarbonate, but we can explore further in the next studies. (Page 9, line 21)

Single measurement of bicarbonate and glucose is also a limitation and should be discussed.
Thanks for this suggestion. We have modified the discussion (Page 9, line 25), and also in the section after abstract.

What was the role of liver ultrasound? It does not seem to be used in any of the analyses.
As mentioned above, we performed ultrasound examinations before and annually during the study to screen for fatty liver disease, liver cysts or tumors. Those participants with abnormal ultrasound results were excluded.

The relevance of ROC analysis is difficult to interpret. One would rarely perform a test for serum bicarbonate and hope to predict future IGT, where there are so many other more significant predictors of developing diabetes, including age or serum glucose level. This is also seen in the relatively low sensitivity and specificity of the test.
Thank you for this comment. The results indicated that low bicarb is a necessary but not sufficient cause to progress to IFG/DM. Notably, the NPV results demonstrated great predictive value for absence of development of IFG. Bicarbonate above a certain level is protective 98% (NPV). (Page 10, line 5)

Correct unit for creatinine should be included in Methods (page 4)
We are sorry for this mistake. The unit has been corrected. (Page 4, line 26)

Please check grammar and spelling.
We have carefully modified the manuscript and asked native speaker help to revise the grammar and spelling.

Reviewer: 2
Reviewer Name: Maria J Redondo
Institution and Country: Baylor College of Medicine, USA
Competing Interests: None declared

Li et al set out to study the relationship between serum bicarb levels and the risk of progression to impaired fasting glucose or diabetes. This research is significant, novel and well conducted, although important mechanistic information is missing. My major comments relate to the analytical methods and presentation of results.

- Subjects and Methods: Please indicate how many participants were still followed at each time point. Also, the schedule of visits is not clearly described and easy to miss by the reader. Please clarify how often, time intervals, number of visits and number (%) of participants at each visit. For each of those questions, please modify the manuscript accordingly.
Thanks for this comments. A total of 12001 Participants were screened for eligibility between January 2006 to December 2006 and 8107 were admitted to this research. From January 2007, participants received telephone call made by research staff every 3 months, required about the plasma glucose, blood pressure, exercise frequency, and medication use et al. Every participant came to the examination center annually for physical examination and questionnaire. Follow-up of the last person was completed in December 2012. From 2007 to 2012, the number of participants at each visit was 7742 (95.5%), 7272 (89.7), 6656 (82.1%), 6081 (75.0%), 5627 (69.4%), 5118 (63.1%), respectively. (Page 4, line 24)

- Related to the above, line 7 indicates "65.6% who completed the follow-up physical examination": Does this refer to finishing all the visits, or being followed by the 6-year time? Please clarify follow-up time and completion rates. Please modify the manuscript accordingly.
After 6-year follow-up, 5318 participants completed all the visits, 1622 withdrew the follow-up, 1139 failed to contact, 28 died. (Page 5, line 7)

- Please compare available characteristics between those who completed follow-up and those who were not. Also, please address if they could have been lost to follow-up due to death or other complications of diabetes, and therefore be a group enriched for progressors. If the latter is true, by analyzing only the ones who did complete follow-up, the actual progression to IFG/DM would be underestimated. Please modify the manuscript accordingly and/or discuss in limitations of the study.
Thanks for this suggestion. It is very important to compare the characteristics between those who completed follow-up and those who were not. However, in our present study, the participants failed to complete the follow-up mainly because of bad communication or compliance, but not due to complications of diabetes or other diseases. Therefore, we did not perform any statistical analysis between these two parts.

- Analytical methods: Why were time-to-event analytical methods not used? Given that the follow-up time was different for the participants, time-to-event methods would have allowed to take this into account and maximize the use of available information. Please explain and modify the analysis accordingly.

Thanks for the suggestion. This is taken seriously. The present research is an observational study without any intervention, the glucose level could be influenced by many different factors, not only serum bicarbonate. Although the follow-up time was different for the participants, we consider it not suitable to do survival analysis. Besides, the incidence of IFG was quite low, below 5%, which may expand the experimental error among the four bicarbonate groups. For these reasons, we performed a logistic regression analysis to evaluate the relationship between serum bicarbonate and the glucose level instead.

- The authors refer to IFG throughout the manuscript while they are referring to IFG or diabetes. This should be made clear to the reader in the text and abstract. Please refer to "IFG or DM" or "IFG/DM" or any other nomenclature or abbreviation that would be clear to the reader what progression meant in this study.
We are grateful for this suggestion. As described in the part “Subjects and Methods”, Page 5 line 12, In this study, participants with an FPG between 3.9-5.5 mmol/L at baseline and with an FPG ≥6.1 mmol/L (including ≥7.0 mmol/L) after follow-up were defined as “progressing to IFG”. A DM patient already passed the status of IFG, and IFG is a pre-diabetic status, the description “risk of progressing to IFG” should include “risk of progressing to DM”.

- Lack of mechanistic information is an important limitation of the study, both for the mechanisms of differences in levels of bicarb among participants, and the effect of bicarb on progression. Is there any addtitional information that could help lessen this limitation? For example, other markers of pancreatic insufficiency?
Thank you for underlining this deficiency. The potential mechanism of the effect of bicarbonate on progression to IFG would be very complicated. One possible explanation, as mentioned in the manuscript, is an upregulation of cystic fibrosis transmembrane conductance regulator (CFTR), which was proved to modulate the glucose-induced electrical activities and insulin secretion in pancreatic β-cells. It is well known that the exocrine and endocrine part of the pancreas interacts with each other, the exocrine insufficiency leads to the dysfunction of endocrine islets. Recently, Fortunato et al. demonstrated autophagy and necroptosis signaling were also involved in the exocrine insufficiency, suggesting that new signaling pathway might participate in the regulation of islet glucose metabolism. (Page 9, line 15)

- why was dyastolic pressure not included in the models along with systolic? If there is any analytical issue, can mean blood pressure be used instead for adjustment? Why were lipids not included? In general, how were the variables selected to be included or excluded from the multivariable analyses? Please explain in your response, clarify the methods in the manuscript and modify the analysis accordingly.
We considered those variables possibly related to blood glucose and bicarbonate level as potential confounders for adjustment. First we performed logistic regression analysis between bicarbonate and each confounder separately to obtain crude OR value, the factors whose $P < 0.2$ in this step were selected to make further adjustments. (Page 6, line 1)

- Results; Please provide the distribution of bicarb levels (median, mean, SD, IQR, range) in your study population as well as the normal reference values in your lab.
As for the reviewer’s concern, the distribution of bicarbonate levels of the participants at baseline is described as follows: mean ± sd 26.71 ± 2.20, median (IQR) 26.80 (25.30-28.42), range (20.1-31.0) mmol/L, the normal reference values in our lab for serum bicarbonate is 24.2-30.7 mmol/L. (Page 6, line 21)

- Please offer plausible explanations for the lower bicarb levels in individuals with lower FPG, serum creat, TC, TG and LDL at baseline.

Thank you for this comment. We have noticed this unexpected relationship. The results in Table 1 were obtained in 5318 participants at baseline, where there is a bias on sex distribution (Male 44%). A total of 8107 participants were admitted in the beginning, but more than 2000 withdrew or lost to follow up. We made another statistical analysis of these characteristics for all the 8107 participants (Male 48%), with the same bicarb groups, and found no significant difference. In addition, these biochemical parameters were acquired in a single test, which could expand the experimental error as well. (Page 9, line 23)

- Please include the percentile that 26.1 corresponds to in your distribution.
We have modified the sentence according to this comment. (Page 8, line 4)

- Limitations of the study, besides the ones mentioned, include lack of data to shed light on the mechanism of the association between low bicarb and progression (e.g. no data on insulin resistance markers or insulin secretion) and on why the inter individual differences in bicarb (e.g. other markers of pancreatic insufficiency). Other limitations are the seizable proportion of missed to follow-up participants; adjustment for confounder variables may have been incomplete and this finding needs validation in an independent cohort to prove reproducibility. Please update the limitaition section of the discussion as well as the paragraph right after the abstract.
WE APPRECIATE FOR THIS ILLUMINATING SUGGESTION. The discussion and the paragraph right after the abstract are modified and updated accordingly. Many thanks for this comment. (Page 10, line 11)

- The authors indicate that "data from multicenter" is needed. However, validation could come not only from a multicenter study but from any other independent cohort(s).
The manuscript has been modified accordingly. (Page 10, line 18)

- Discussion: My interpretation of the findings is that low bicarb is a necessary but not sufficient cause to progress to IFG/DM. Bicarb above a certain level is protective 98% (NPV). Are those statements correct? If so, please clarify in the discussion. I find that more remarkable that a mild increase in the risk conferred by lower carb. Please clarify to this reader and modify discussion if appropriate.
We have modified the discussion according to this comment. These statements are consistent with what we meant to express to the reader. As the PPV value is low while the NPV is quite high, it seems that above a certain level bicarbonate is protective, but low bicarbonate is not a sufficient cause to progress to IFG/DM. (Page 10, line 5)

- Where medications considered as a potential confounder? Other potential confounders? Please explain and modify the discussion (i.e. add as limitation) accordingly.
The discussion part has been modified. We consider that dietary especially water/beverage drinking habits influence the glucose metabolism, which was not investigated thoroughly. (Page 10, line 15)

- Discussion needs to explain the baseline associations that were unexpected (e.g. some of them are inverse to the expected).
This question is similar to the ones above, please see the modified manuscript (Page 9, line 23).

- DKA also impairs beta-cell function. This work is consistent with that previous observation. Please add to the discussion if appropriate or explain why is not in your response.
This work is consistent with previous observations, Prof. Redondo demonstrated that insulin deficiency is a major contributor to DKA but, conversely, the metabolic effects of ketoacidosis may temporarily decrease further the ability to secrete insulin. A probable relationship links the low-pH environment and impaired insulin secretion, but the precise mechanism needs more exploration. We have modified the discussion. (Page 8, line 26)

- MINOR
--- The study needs English edits. A few examples are: line 11, page 3: a large 'amount' of participants (please replace with 'number')
This sentence has been modified.
--- Ultrasound is mentioned in line 13, page 3 but not in the paper. Please reconcile. If not analyzed in this study, please delete.
Ultrasound has been deleted.
--- line 15-16, page 3, needs English revision
We have modified this part of limitation.
----there are multiple other instances.
- page 4, line 5: 'intermediate hyperglycemia' is an unusual name. Please consider replacing with 'pre-diabetes'
This phrase has been replaced.
- "the" public health, extra "the" line 7-8, page 4
The extra word has been deleted.
- results, line 43-46, page 6,: Table S1 shows that participants..." is a repeat of previous information.
- Lines 53-54, page 8,: "Moreover, fasting hyperglycemia..." Please clarify the meaning of this sentence and rephrase accordingly in the manuscript.
- Line 17-20 , page 10: the sentence "although HbA1c determination..." is unclear to this reader. Please clarify in your response and rephrase accordingly in the manuscript.
These sentences have all been deleted.

VERSION 2 – REVIEW

REVIEWER	MJ Redondo Baylor College of Medicine, Texas Children's Hospital, USA
REVIEW RETURNED	06-Nov-2017

GENERAL COMMENTS	Li et al have made changes to their manuscript in response to reviewers' comments. However, some questions remain: ORIGINAL COMMENT- Please compare available characteristics between those who completed follow-up and those who were not. Also, please address if they could have been lost to follow-up due to death or other complications of diabetes, and therefore be a group enriched for progressors. If the latter is true, by analyzing only the ones who did complete follow-up, the actual progression to IFG/DM would be underestimated. Please modify the manuscript accordingly and/or discuss in limitations of the study. AUTHORS' RESPONSE: Thanks for this suggestion. It is very important to compare the characteristics between those who completed follow-up and those who were not. However, in our present study, the participants failed to complete the follow-up mainly because of bad communication or compliance, but not due to complications of diabetes or other diseases. Therefore, we did not perform any statistical analysis between these two parts. REVIEWER'S COMMENT: A comparison between those who dropped out and those who continued in the study is very important for an analysis with many potential confounders. Of course, this analysis would be limited to the data that is available for those who dropped off. For instance, from the authors' response, this reviewer concludes that they have information on progression to diabetes in those who dropped off. It would be most interesting to include that information as well, and compare with those who remained in the study.

	ORIGINAL COMMENT- Analytical methods: Why were time-to-event analytical methods not used? Given that the follow-up time was different for the participants, time-to-event methods would have allowed to take this into account and maximize the use of available information. Please explain and modify the analysis accordingly. AUTHORS' RESPONSE: Thanks for the suggestion. This is taken seriously. The present research is an observational study without any intervention, the glucose level could be influenced by many different factors, not only serum bicarbonate. Although the follow-up time was different for the participants, we consider it not suitable to do survival analysis. Besides, the incidence of IFG was quite low, below 5%, which may expand the experimental error among the four bicarbonate groups. For these reasons, we performed a logistic regression analysis to evaluate the relationship between serum bicarbonate and the glucose level instead. REVIEWER'S RESPONSE: I recommend peer review by biostatistician to ensure this is the correct analysis. ORIGINAL COMMENT- The authors refer to IFG throughout the manuscript while they are referring to IFG or diabetes. This should be made clear to the reader in the text and abstract. Please refer to "IFG or DM" or "IFG/DM" or any other nomenclature or abbreviation that would be clear to the reader what progression meant in this study. AUTHORS' RESPONSE: We are grateful for this suggestion. As described in the part “Subjects and Methods”, Page 5 line 12, In this study, participants with an FPG between 3.9-5.5 mmol/L at baseline and with an FPG ≥6.1 mmol/L (including ≥7.0 mmol/L) after follow-up were defined as “progressing to IFG”. A DM patient already passed the status of IFG, and IFG is a pre-diabetic status, the description “risk of progressing to IFG” should include “risk of progressing to DM”. REVIEWER'S COMMENT: My impression is that readers will be confused by the name IFG when referring to diabetes. If this study is analyzing both pre-diabetes and/or diabetes, it should state that clearly throughout the manuscript. ORIGINAL COMMENT- Lack of mechanistic information is an important limitation of the study, both for the mechanisms of differences in levels of bicarb among participants, and the effect of bicarb on progression. Is there any addtitional information that could help lessen this limitation? For example, other markers of pancreatic insufficiency? AUTHORS'S RESPONSE Thank you for underlining this deficiency. The potential mechanism of the effect of bicarbonate on progression to IFG would be very complicated. One possible explanation, as mentioned in the manuscript, is an upregulation of cystic fibrosis transmembrane conductance regulator (CFTR), which was proved to modulate the glucose-induced electrical activities and insulin secretion in pancreatic β-cells. It is well known that the exocrine and endocrine part of the pancreas interacts with each other, the exocrine insufficiency leads to the dysfunction of endocrine islets. Recently, Fortunato et al. demonstrated autophagy and necroptosis signaling were also involved in the exocrine insufficiency, suggesting that new signaling pathway might participate in the regulation of islet

	glucose metabolism. (Page 9, line 15) REVIEWER'S COMMENT: Lack of mechanistic data to support biological plausibility should be clearly mentioned among the limitations of the study. ORIGINAL COMMENT- Where medications considered as a potential confounder? Other potential confounders? Please explain and modify the discussion (i.e. add as limitation) accordingly. AUTHOR'S RESPONSE The discussion part has been modified. We consider that dietary especially water/beverage drinking habits influence the glucose metabolism, which was not investigated thoroughly. (Page 10, line 15) REVIEWER'S COMMENT: How about medications that may change the pH? This should also be included in the discussion as a limitation, along with dietary differences.

REVIEWER	Mirela Dobre UHCMC
REVIEW RETURNED	04-Dec-2017

GENERAL COMMENTS	Sen Li et al. aim to investigate the association between serum bicarbonate and the risk of progressing to impaired fasting glucose (IFG). Few comments below: Major: 1. Bicarbonate at levels above 28 mmol/L can be detrimental too. A U-shaped association between serum bicarbonate levels and mortality has been shown in various studies of CKD or non-CKD, therefore the data should be interpreted with caution. Results replication is needed in a multicenter study. 2. The association between lower bicarbonate and low creatinine is counterintuitive and should be revised/re-analyzed and potential explanation should be provided. Same is true for FPG: the low bicarbonate group had the lowest FPG, however this group was the one most likely to progress to IFG. How do authors interpret these findings? 3. It is unclear what type of regression analyses were used. If survival analyses, then hazard ratios should be calculated. 4. Study limitations should be expanded to include: single measurement of bicarbonate, lack of available serum pH, not available dietary intake, proteinuria, single center, Asian population, etc 5. Authors needs a better description of population and methods, including comorbidities, dietary intake that can significantly affect the results, medication data: diuretics, antiacid medications, etc. Was the FPG measured annually only during study visits or also ambulatory every 3 months? 6. More discussion about potential mechanisms linking bicarbonate with insulin resistance is needed Minor: 1. Table 2 is self-explanatory and the wording in the results section pertaining to data in Table 2 can be significantly shorten.

	2. Creatinine level cut-off chosen can still represent CKD for some elderly patients with a low muscle mass. 3. Check spelling and grammar

REVIEWER	Jian Liu Brock University, Canada
REVIEW RETURNED	05-Jan-2018

GENERAL COMMENTS	Comments on bmjopen-2017-019145.R1 "Is serum bicarbonate associated with the risk of progressing to impaired fasting glucose? A single-center prospective cohort study" Using data generated from 5318 people who took physical examination at a hospital, authors examined the risk association of IFG with serum bicarbonate level and concluded that Lower serum bicarbonate is associated with higher risk of the development of IFG. Below are the reviewer's comments, which I put into two categories, major vs. minor concerns: Major concerns: 1. Participants. Based on methods, 8107 participants aged 18-70 years were eligible for the study, "from 2007 to 2012, the number of participants at each visit was 7742 (95.5%), 7272 (89.7), 6656 (82.1%), 6081 (75.0%), 5627 (69.4%), 5118 (63.1%), respectively. After 6 years follow-up, 5318 participants completed all the visits" It doesn't make any sense that if the # for the last visiting was 5118, there were 5318 people who completed all 6 annually visits. 2. Study design. As a cohort study, there was no description regarding how those individuals were sampled, no information for other demographic (eg, marriage status, education, etc.), lifestyle (eg, smoking, alcohol, activity, etc.), disease history, family history, etc. was this a cohort with special health conditions? There were no discussions on its generalizability. Table 1 listed hypertension as a disease, but there was no information of the definition of the disease. 3. Laboratory procedure. How was physical examination organized for each person? Was there any lab protocol to follow? Eg, how many hrs was required for fasting? Morning blood sample? The BMI and BP information was listed in the tables, but it was surprised that there were no description of the methods for their measurements. Were they self-reported? 4. Analyses. a) All continuous variables listed on table 1 were medians with IQR. No idea why do that? b) Was there a linear relationship between the levels of serum bicarbonate and of FPG both as continuous variables? As the first study to evaluate the relationship between serum bicarbonate and the risk of progressing to impaired fasting glucose, it is worth to explore whether these two are linear correlated. c) also how was bicarbonate level as a continuous variable for the outcome? d) while the hypertension proportions were so different, why not adjusting for hypertension? e) since FIG was defined using FPG, no necessary to adjust for the level of FPG; f) no idea for "Participant subgroups were categorized by age, sex, BMI, SBP and LDL-C." in pg 7 line49; g) no idea what ORs in figure 1 mean. Eg, age >30 vs. <=30, which one is the reference? Based on table 1, the older people seemed more likely to be in low level bicarbonate quartile, why in figure 1, older age showed a lower OR than those youngers? H) in table s2, where were those with serum bicarbonate = 26.15? Minors: 5. Title. Should be"Are the low levels of serum bicarbonate associated with the risk of progressing to impaired fasting glucose?"

	more appropriate? 6. Table 1, the cut-offs of bicarbonate were not clear. Quartile 1(<25.8), (25.8—26.8), (26.8—28.0), and Quartile 4 (>28.0). If 25.8 was belong to Q2, then 28.0 should belong to the Q4 as well. 7. Decimal points should be the same for the same measurements. Eg, pg 6, line 44-49 “The bicarbonate level analyzed ranges from 20.1-31.0 mmol/L, with mean ± sd (26.71 ± 2.20) mmol/L, median (IQR) 26.80 (25.30-28.42) mmol/L, the normal reference values in our lab for serum bicarbonate is 24.2-30.7 mmol/L” 8. For tables s1-2, there were only two categories, ie, low vs. high. Why was the title quartiles of …?

VERSION 2 – AUTHOR RESPONSE

Reviewers' Comments to Author:

Reviewer: 1

Reviewer Name: Mirela Dobre

Institution and Country: UHCMC

Competing Interests: none

Sen Li et al. aim to investigate the association between serum bicarbonate and the risk of progressing to impaired fasting glucose (IFG). Few comments below:

Major:

1. Bicarbonate at levels above 28 mmol/L can be detrimental too. A U-shaped association between serum bicarbonate levels and mortality has been shown in various studies of CKD or non-CKD, therefore the data should be interpreted with caution. Results replication is needed in a multicenter study.

Thanks for this valuable comments, we have revised the manuscript accordingly (Page 9, Line 26). The single-center research is limited and these results need replication in a multicenter study, which has been clearly written in the Discussion part. We searched more literatures on the association between serum bicarbonate levels and mortality. Prof. Dobre et al. demonstrated that in CKD patients, persistent serum bi-carbonate >26mmol/L was associated with increased risk of heart failure events and mortality. However, in another study among NHANES III participants, low serum bicarbonate was not observed to be a strong predictor of mortality in people without CKD. The present non-CKD research is not contradicted with these two studies, but we do adopt the reviewer’s comments, our data should be interpreted with caution.

2. The association between lower bicarbonate and low creatinine is counterintuitive and should be revised/re-analyzed and potential explanation should be provided. Same is true for FPG: the low bicarbonate group had the lowest FPG, however this group was the one most likely to progress to IFG. How do authors interpret these findings?

Thanks for this comments. We have noticed this unexpected relationship. The results in Table 1 were obtained in 5318 participants at baseline, where there is a bias on sex distribution (Male 44%). A total of 8107 participants were admitted in the beginning, but more than 2000 withdrew or lost to follow up. We made another statistical analysis of these characteristics for all the 8107 participants (Male 48%), and found no significant difference of serum creatinine or FPG among different bicarbonate groups. In addition, these biochemical parameters were acquired in a single test, which could expand the experimental error as well. (Page 9, line 23)

3. It is unclear what type of regression analyses were used. If survival analyses, then hazard ratios should be calculated.

As we described in the Statistical Analysis part, logistic regression was used in this research. We have consulted biostatistics experts, 210 participants developed IFG in a total of 5318, and 15 variables were included in this observation, the final events per variable is quite small, the accuracy and precision would be affected in a survival analysis. Actually, we tried a Cox regression for this study, we set the highest quartile of bicarbonate as reference, the hazard ratios of the other groups from low to high bicarb were 1.56, 1.25, 1.08, respectively.

4. Study limitations should be expanded to include: single measurement of bicarbonate, lack of available serum pH, not available dietary intake, proteinuria, single center, Asian population, etc.

Thanks for this valuable comments. The limitations were expanded as descripted in the Discussion, please check (Page 10, Line 15).

5. Authors needs a better description of population and methods, including comorbidities, dietary intake that can significantly affect the results, medication data: diuretics, antiacid medications, etc. Was the FPG measured annually only during study visits or also ambulatory every 3 months?

This comment is very important and helps to improve our work. The population and methods part has been revised accordingly. Please check Page 4, Line 26. Also, the FPG was measured not only annually during study visits, but also ambulatory every 3 months.

6. More discussion about potential mechanisms linking bicarbonate with insulin resistance is needed.

Thanks for this suggestion. Potential mechanisms on the insulin resistance has been updated in the discussion, please check Page 8, Line 20.

Minor:

1. Table 2 is self-explanatory and the wording in the results section pertaining to data in Table 2 can be significantly shorten.

The wording in the results section pertaining to data in Table 2 has been significantly shortened.

2. Creatinine level cut-off chosen can still represent CKD for some elderly patients with a low muscle mass.

The participants over 60 years old with low muscle mass were excluded to this study.

3. Check spelling and grammar

Reviewer: 2

Reviewer Name: MJ Redondo

Institution and Country: Baylor College of Medicine, Texas Children's Hospital, USA

Competing Interests: None declared

Li et al have made changes to their manuscript in response to reviewers' comments. However, some questions remain:

ORIGINAL COMMENT- Please compare available characteristics between those who completed follow-up and those who were not. Also, please address if they could have been lost to follow-up due to death or other complications of diabetes, and therefore be a group enriched for progressors. If the latter is true, by analyzing only the ones who did complete follow-up, the actual progression to IFG/DM would be underestimated. Please modify the manuscript accordingly and/or discuss in limitations of the study.

AUTHORS' RESPONSE: Thanks for this suggestion. It is very important to compare the characteristics between those who completed follow-up and those who were not. However, in our present study, the participants failed to complete the follow-up mainly because of bad communication or compliance, but not due to complications of diabetes or other diseases. Therefore, we did not perform any statistical analysis between these two parts.

REVIEWER'S COMMENT: A comparison between those who dropped out and those who continued in the study is very important for an analysis with many potential confounders. Of course, this analysis would be limited to the data that is available for those who dropped off. For instance, from the authors' response, this reviewer concludes that they have information on progression to diabetes in those who dropped off. It would be most interesting to include that information as well, and compare with those who remained in the study.

Thanks for this valuable comments. The demographic and laboratory data were both compared between those who finished and those who dropped out the study, and there was no statistically significant difference.

ORIGINAL COMMENT- Analytical methods: Why were time-to-event analytical methods not used? Given that the follow-up time was different for the participants, time-to-event methods would have allowed to take this into account and maximize the use of available information. Please explain and modify the analysis accordingly.

AUTHORS' RESPONSE: Thanks for the suggestion. This is taken seriously. The present research is an observational study without any intervention, the glucose level could be influenced by many different factors, not only serum bicarbonate. Although the follow-up time was different for the participants, we consider it not suitable to do survival analysis. Besides, the incidence of IFG was quite low, below 5%, which may expand the experimental error among the four bicarbonate groups. For these reasons, we performed a logistic regression analysis to evaluate the relationship between serum bicarbonate and the glucose level instead.

REVIEWER'S RESPONSE: I recommend peer review by biostatistician to ensure this is the correct analysis.

We also have consulted biostatistics experts, in this study, 210 participants developed IFG/DM in a total of 5318, and 15 variables were included in this observation, the final events per variable is quite small, the accuracy and precision would be affected in a survival analysis. Actually, we made a Cox regression for this study, the highest quartile of bicarbonate was set as reference, the hazard ratios of the other groups from low to high bicarb were 1.56, 1.25, 1.08, respectively. Nevertheless, we will follow the final decision of the reviewers and the chief editor.

ORIGINAL COMMENT- The authors refer to IFG throughout the manuscript while they are referring to IFG or diabetes. This should be made clear to the reader in the text and abstract. Please refer to "IFG or DM" or "IFG/DM" or any other nomenclature or abbreviation that would be clear to the reader what progression meant in this study.

AUTHORS' RESPONSE: We are grateful for this suggestion. As described in the part “Subjects and Methods”, Page 5 line 12, In this study, participants with an FPG between 3.9-5.5 mmol/L at baseline and with an FPG ≥6.1 mmol/L (including ≥7.0 mmol/L) after follow-up were defined as “progressing to IFG”. A DM patient already passed the status of IFG, and IFG is a pre-diabetic status, the description “risk of progressing to IFG” should include “risk of progressing to DM”.

REVIEWER'S COMMENT: My impression is that readers will be confused by the name IFG when referring to diabetes. If this study is analyzing both pre-diabetes and/or diabetes, it should state that clearly throughout the manuscript.

Thanks for your suggestion. We revised all the description in the previous manuscripts, the name “IFG” was replaced by “IFG/DM”.

ORIGINAL COMMENT- Lack of mechanistic information is an important limitation of the study, both for the mechanisms of differences in levels of bicarb among participants, and the effect of bicarb on progression. Is there any addtitional information that could help lessen this limitation? For example, other markers of pancreatic insufficiency?

AUTHORS'S RESPONSE Thank you for underlining this deficiency. The potential mechanism of the effect of bicarbonate on progression to IFG would be very complicated. One possible explanation, as mentioned in the manuscript, is an upregulation of cystic fibrosis transmembrane conductance

regulator (CFTR), which was proved to modulate the glucose-induced electrical activities and insulin secretion in pancreatic β-cells. It is well known that the exocrine and endocrine part of the pancreas interacts with each other, the exocrine insufficiency leads to the dysfunction of endocrine islets. Recently, Fortunato et al. demonstrated autophagy and necroptosis signaling were also involved in the exocrine insufficiency, suggesting that new signaling pathway might participate in the regulation of islet glucose metabolism. (Page 9, line 15)

REVIEWER'S COMMENT: Lack of mechanistic data to support biological plausibility should be clearly mentioned among the limitations of the study.

Thanks for this comment. Lack of mechanistic data has been clearly mentioned in the “Strength and Limitation” and Discussion Part.

ORIGINAL COMMENT- Where medications considered as a potential confounder? Other potential confounders? Please explain and modify the discussion (i.e. add as limitation) accordingly.

AUTHOR'S RESPONSE The discussion part has been modified. We consider that dietary especially water/beverage drinking habits influence the glucose metabolism, which was not investigated thoroughly. (Page 10, line 15)

REVIEWER'S COMMENT: How about medications that may change the pH? This should also be included in the discussion as a limitation, along with dietary differences.

Thanks for the suggestion. Medications that may change the pH have been included in the discussion as well.

Reviewer: 3

Reviewer Name: Jian Liu

Institution and Country: Brock University, Canada

Competing Interests: none

Using data generated from 5318 people who took physical examination at a hospital, authors examined the risk association of IFG with serum bicarbonate level and concluded that Lower serum bicarbonate is associated with higher risk of the development of IFG. Below are the reviewer’s comments, which I put into two categories, major vs. minor concerns:

Major concerns:

1. Participants. Based on methods, 8107 participants aged 18-70 years were eligible for the study, “from 2007 to 2012, the number of participants at each visit was 7742 (95.5%), 7272 (89.7), 6656 (82.1%), 6081 (75.0%), 5627 (69.4%), 5118 (63.1%), respectively. After 6 years follow-up, 5318 participants completed all the visits” It doesn’t make any sense that if the # for the last visiting was 5118, there were 5318 people who completed all 6 annually visits.

Thanks for this comment. The number “7742, 7272, 6656, 6081, 5627, 5118” is the number of participants who were still at follows at the end of each year from 2007 to 2012 , excluding those who had already reached IFG/DM, and those who dropped out the research. The number “5318” includes “5118” and the number of participants who had progressed to IFG/DM during the entire observation.

2. Study design. As a cohort study, there was no description regarding how those individuals were sampled, no information for other demographic (eg, marriage status, education, etc.), lifestyle (eg, smoking, alcohol, activity, etc.), disease history, family history, etc. was this a cohort with special health conditions? There were no discussions on its generalizability. Table 1 listed hypertension as a disease, but there was no information of the definition of the disease.

Thanks for this valuable comment. The study design in the Method Part has been revised accordingly. Participants were randomly sampled and major excluding criteria has been descripted in “Page 4, Line 26” Demographic and lifestyle variables including marriage status, nationality and smoking have been added to Table 1. The generalizability of this study was discussed in Page 10, Line 14.

The definition of hypertension has been revised in Page, Line.

3. Laboratory procedure. How was physical examination organized for each person? Was there any lab protocol to follow? Eg, how many hrs was required for fasting? Morning blood sample? The BMI and BP information was listed in the tables, but it was surprised that there were no description of the methods for their measurements. Were they self-reported?

Thanks for this comment. The laboratory procedure was revised accordingly.

4. Analyses. a) All continuous variables listed on table 1 were medians with IQR. No idea why do that?

Like mean and standard deviation, median and IQR measure the central tendency and spread, respectively, but are robust against outliers and non-normal data. They have a couple of additional advantages:

Outlier Identification. IQR makes it easy to do an initial estimate of outliers by looking at values more than one-and-a-half times the IQR distance below the first quartile or above the third quartile.

Skewness. Comparing the median to the quartile values shows whether data is skewed.

Based on the theories above, we use medians with IQR in the study.

b) Was there a linear relationship between the levels of serum bicarbonate and of FPG both as continuous variables? As the first study to evaluate the relationship between serum bicarbonate and the risk of progressing to impaired fasting glucose, it is worth to explore whether these two are linear correlated.

Thanks for this suggestion. We have made a linear regression analysis but find no significant relationship between serum bicarbonate and FPG.

c) also how was bicarbonate level as a continuous variable for the outcome?

Thanks for this comment. It is not the LEVEL of bicarbonate descripted as a continuous variable, but the concentration of serum bicarbonate.

d) while the hypertension proportions were so different, why not adjusting for hypertension?

We have noticed the high proportion of hypertension in the first quartile participants, and SBP has been already adjusted in the manuscript, please check the statistical method part (Page 6, Line 11-17).

e) since FIG was defined using FPG, no necessary to adjust for the level of FPG;

Thanks for this suggestion. Although IFG was defined using FPG, it is not contradictory to evaluate the relationship between the initial level of FPG at the baseline and the risk of progressing to IFG in a few years.

f) no idea for “Participant subgroups were categorized by age, sex, BMI, SBP and LDL-C.” in pg 7 line49;

g) no idea what ORs in figure 1 mean. Eg, age >30 vs. <=30, which one is the reference? Based on table 1, the older people seemed more likely to be in low level bicarbonate quartile, why in figure 1, older age showed a lower OR than those youngers?

Thanks for these comments. We feel sorry for making this figure unclear. First we performed logistic regression analysis between bicarbonate and each confounder separately to obtain crude OR value, the factors whose $P < 0.2$ in this step were selected to make further adjustments. The variables in comment f) and g) were thus chosen to make subgroups. The ORs were not compared between these subgroups, as we described in the figure legend, they were between the individuals with serum bicarbonate below the median level and those above the median level in each subgroup. The higher ones are references. However, considering that this may lead to readers’ misunderstanding or confusion, we decide to delete this figure.

H) in table s2, where were those with serum bicarbonate = 26.15?

Thanks for points this mistake. The group of serum bicarbonate ≥26.15 was set as reference.

Minors:

5. Title. Should be”Are the low levels of serum bicarbonate associated with the risk of progressing to impaired fasting glucose?” more appropriate?

The title has been revised accordingly.

6. Table 1, the cut-offs of bicarbonate were not clear. Quartile 1(<25.8), (25.8—26.8), (26.8—28.0), and Quartile 4 (>28.0). If 25.8 was belong to Q2, then 28.0 should belong to the Q4 as well.

Thanks for this comments. Quartile 4 has been revised as “≥28.0”.

7. Decimal points should be the same for the same measurements. Eg, pg 6, line 44-49 “The bicarbonate level analyzed ranges from 20.1-31.0 mmol/L, with mean ± sd (26.71 ± 2.20) mmol/L, median (IQR) 26.80 (25.30-28.42) mmol/L, the normal reference values in our lab for serum bicarbonate is 24.2-30.7 mmol/L”

We are sorry for this mistake. Decimal points have been revised to be the same for the same measurements.

8. For tables s1-2, there were only two categories, ie, low vs. high. Why was the title quartiles of …?

Thanks for pointing this mistake. The titles of table s1-2 have been revised.

VERSION 3 – REVIEW

REVIEWER	Jian Liu Brock University, Canada
REVIEW RETURNED	19-Feb-2018

GENERAL COMMENTS	1. I'm satisfy the explanation on authors' responses to the # of "7742, 7272, 6656, 6081, 5627, 5118" in page 5 lines 10 – 13, but it is still confusing for readers. I'd suggest adding those who have been excluded due to having reached IFG/DM to make the final # of cohort as 5318. Or specify that these # didn't include those who reached IFG/DM. is this right 5118 without IFG/DM +200 with IFG/DM developed during the follow up? This number seems not match with what described in results, n=210. By the way, should those DM be most likely type 2 diabetes? 2. Fasting blood for glucose test usually needs at least 8 hrs overnight fast. How many people did meet the minimum requirement? How likely did this affect the IFG/DM cases diagnosis (if they were excluded)? Should the potential bias be discussed? 3. Was ROC AUC .69 by serum bicarbonate alone? Usually, ROC AUC <0.7 is considered as poor discrimination. Should this be discussed? 4. The 1st sentence in discussion "In this study, we demonstrated that low level of serum bicarbonate increased the prevalence of IFG/DM independent of risk factors" needs to be revised. Probably, "observed" is more appropriate here than "demonstrated". 5. Non-parametric methods are much appreciated being used in small sample sized data and uncertainty of normal distribution. Table 1 should be reanalyzed with parametric methods since each category has a large sample size.

REVIEWER	MJ Redondo,MD Baylor College of Medicine, USA
REVIEW RETURNED	28-Feb-2018

GENERAL COMMENTS	The authors have responded to my questions. I have no further questions.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 2
Reviewer Name: MJ Redondo,MD
Institution and Country: Baylor College of Medicine, USA
Competing Interests: None

The authors have responded to my questions. I have no further questions.

Reviewer: 3
Reviewer Name: Jian Liu
Institution and Country: Brock University, Canada
Competing Interests: none

1. I'm satisfy the explanation on authors' responses to the # of "7742, 7272, 6656, 6081, 5627, 5118" in page 5 lines 10 – 13, but it is still confusing for readers. I'd suggest adding those who have been excluded due to having reached IFG/DM to make the final # of cohort as 5318. Or specify that these # didn't include those who reached IFG/DM. is this right 5118 without IFG/DM +200 with IFG/DM developed during the follow up? This number seems not match with what described in results, n=210. By the way, should those DM be most likely type 2 diabetes?
Thanks for this suggestion. We accepted the reviewer's comment, and have specified in the manuscript that these numbers did not include those who had reached IFG/DM. Although we do not have laboratory data on the insulin secretion or beta-cell function, those DM are most likely to be T2DM according to their clinical features and physical examinations.

2. Fasting blood for glucose test usually needs at least 8 hrs overnight fast. How many people did meet the minimum requirement? How likely did this affect the IFG/DM cases diagnosis (if they were excluded)? Should the potential bias be discussed?
Thanks for this comment. To make sure at least 8 hours overnight fast, each participant was informed not to eat or drink after 10pm before the test and the blood samples were collected at 7am the next morning. Those who did not meet this requirement were refused to make blood sampling and suggested for another test later. Therefore, all the people meet the minimum requirement.

3. Was ROC AUC .69 by serum bicarbonate alone? Usually, ROC AUC <0.7 is considered as poor discrimination. Should this be discussed?
Thanks for this valuable comment. The AUC was calculated by serum bicarbonate alone. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system:
.90-1 = excellent (A)
.80-.90 = good (B)
.70-.80 = fair (C)
.60-.70 = poor (D)
.50-.60 = fail (F)
We revised the discussion part as follows: The AUC of the ROC curve of serum bicarbonate was 0.69, which indicates a poor discrimination for serum bicarbonate of predicting IFG/DM. However, the 97.8% NPV results demonstrated great predictive value for the absence of development of IFG/DM. Bicarbonate above a certain level is protective. The optimum cut-off value for predicting progression to IFG/DM was 26.1 mmol/L for serum bicarbonate, which was very close to the median level. The mechanism of progressing to IFG/DM is complicated and should not be predicted by a single parameter. Low bicarbonate is a necessary but not sufficient cause of progression to IFG/DM.
4. The 1st sentence in discussion "In this study, we demonstrated that low level of serum bicarbonate increased the prevalence of IFG/DM independent of risk factors" needs to be revised. Probably, "observed" is more appropriate here than "demonstrated".
We accept this suggestion and have changed the word to "observed".
5. Non-parametric methods are much appreciated being used in small sample sized data and uncertainty of normal distribution. Table 1 should be reanalyzed with parametric methods since each category has a large sample size.
Thanks for this statistical suggestion. The measurement data in Table 1 has been reanalyzed with one-way ANOVA method and new P values were calculated as well.

VERSION 4 – REVIEW

REVIEWER	Jian Liu Brock University, Canada
REVIEW RETURNED	17-Apr-2018

GENERAL COMMENTS

Review comments on bmjopen-2017-019145.R3 "**Are low levels of serum bicarbonate associated with risk of progressing to impaired fasting glucose/diabetes? A single-center prospective cohort study in Beijing, China"**

I'm satisfied the changes, but still consider that the table 1 needs to be modified and all continuous variables in the table should be presented as mean (SD) instead of median (IQR). I made examples with Age and BMI. For P-values, I would suggest to have four categories and footnote the symbols, ie, * for <.05, ** for <.01, *** for <.001, and NS for no significance.

Table 1: Baseline characteristics of participants by quartile of serum bicarbonate

Characteristics	**Serum bicarbonate(mmol/L)**				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**P-value**
	(<25.8)	(25.8—26.8)	(26.8—28.0)	(≥28.0)	
n	1340	1426	1322	1230	
Male, %	47.1	43.1	39.9	43.3	0.003
Age (years, mean [SD])	42 (31-50)	32 (26-39)	29 (26-36)	29 (26-39)	<0.001
Married, %	85.7	86.1	84.2	84.0	0.002
Han-nationality, %	90.3	91.2	91.4	90.6	0.002
Current smoking, %	15.6	14.7	14.3	15.0	<0.001
Body mass index (kg/m^2, mean [SD])	23.5 (21.4-26.0)	22.3 (20.2-24.7)	22.3 (20.2-24.7)	22.3 (20.3-24.9)	0.001
Hypertension,	4.8	3.1	0.9	1.5	<0.00

	%					1
	Systolic blood pressure, mmHg	110 (100-120)	110 (100-120)	110 (100-120)	110 (100-120)	0.05
	Diastolic blood pressure, mmHg	80 (70-85)	70 (70-80)	70 (65-75)	70 (65-80)	<0.001
	ALT, U/L	17 (13-25)	17 (13-25)	18 (14-26)	18 (14-26)	0.005
	AST, U/L	25 (21-30)	24 (20-29)	24 (20-29)	24 (20-29)	0.03
	Serum creatinine, μmol/L	65 (57-78)	66 (57-79)	67 (58-80)	70 (60-82)	<0.001
	Fasting blood glucose, mmol/L	5.0 (4.8-5.2)	5.1 (4.8-5.3)	5.1 (4.8-5.3)	5.1 (4.9-5.3)	0.02
	Total cholesterol, mmol/L	4.6 (4.0-5.2)	4.6 (4.1-5.2)	4.7 (4.2-5.3)	4.7 (4.2-5.3)	0.01
	Triglyceride, mmol/L	0.9 (0.7-1.4)	1.0 (0.7-1.5)	1.0 (0.7-1.5)	1.1 (0.8-1.6)	0.03
	HDL, mmol/L	1.5 (1.2-1.7)	1.5 (1.2-1.7)	1.5 (1.2-1.7)	1.5 (1.2-1.7)	0.33
	LDL, mmol/L	2.8 (2.3-3.3)	2.8 (2.4-3.3)	2.8 (2.4-3.4)	2.9 (2.4-3.4)	0.04
	Serum bicarbonate, mmol/L	25.3 (23.9-25.6)	26.4 (26.1-26.6)	27.5 (27.1-27.8)	28.8 (28.4-29.3)	<0.001

Note: HDL= high density lipoprotein, LDL= low density lipoprotein, ALT= alanine aminotransferase, AST= aspartate aminotransferase, IQR= interquartile range.

P values are from one-way ANOVA for those reported as medians and χ^2 test for those reported as percentages.

Data are ~~median (IQR) unless otherwise indicated~~.

Comment [JL1]: Should be mean (SD)

VERSION 4 – AUTHOR RESPONSE

Reviewer's Comments to Author:
Reviewer: 3
Reviewer Name: Jian Liu
Institution and Country: Brock University, Canada
Competing Interests: none declared

comments attached

Thanks for these valuable comments. All continuous variables in Table 1 have been revised to be presented as mean (SD) instead of median (IQR). Besides, the P values were divided into four categories and footnoted with symbols as suggested.